

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION



The T Allele of the Hepatic Lipase Promoter Variant C-480T Is Associated With Increased Fasting Lipids and HDL and Increased Preprandial and Postprandial LpCIII:B : European Atherosclerosis Research Study (EARS) II

Hans Jansen, Grace Chu, Christian Ehnholm, Jean Dallongeville, Viviane Nicaud and Philippa J. Talmud

Arterioscler. Thromb. Vasc. Biol. 1999;19;303-308

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association.
7272 Greenville Avenue, Dallas, TX 75214

Copyright © 1999 American Heart Association. All rights reserved. Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://atvb.ahajournals.org/cgi/content/full/19/2/303>

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at
<http://atvb.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, 351 West Camden Street, Baltimore, MD 21202-2436. Phone 410-5280-4050. Fax: 410-528-8550. Email:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
<http://www.lww.com/static/html/reprints.html>

The *T* Allele of the Hepatic Lipase Promoter Variant C-480T Is Associated With Increased Fasting Lipids and HDL and Increased Preprandial and Postprandial LpCIII:B

European Atherosclerosis Research Study (EARS) II

Hans Jansen, Grace Chu, Christian Ehnholm, Jean Dallongeville, Viviane Nicaud, Philippa J. Talmud, for the EARS Group

Abstract—The common C-480T transition in the hepatic lipase (HL) promoter has been shown to be associated with lower HL activity and increased high density lipoprotein (HDL) cholesterol. We examined the frequency and lipid associations of this HL polymorphism in 385 healthy, young (18- to 28-year-old) men whose fathers had had a premature myocardial infarction (designated cases) and 405 age-matched controls. These individuals were participants in the European Atherosclerosis Research Study II postprandial trial, who had been recruited from 11 European countries in 4 regions (the Baltic; United Kingdom; and central and southern Europe). Overall, the frequency of the *T* allele was 0.207 in controls and 0.244 in cases ($P=0.08$). The *T* allele was associated with higher fasting plasma total cholesterol ($P<0.01$), triglycerides ($P<0.01$), and HDL cholesterol ($P<0.01$). The strongest association was found with apolipoprotein (apo) A-I concentration, which was 10% higher in individuals homozygous for the *T* allele compared with those homozygous for the *C* allele ($P<0.001$). This polymorphism had no effect on the rise in plasma triglyceride levels after a fatty meal. However, before and after the fat load was ingested, levels of particles containing both apoC-III and apoB (LpC-III:B) were higher in carriers of the *T* allele, with homozygotes having 23% and 27% higher levels preprandially and postprandially, respectively, than those homozygous for the *C* allele ($P<0.05$). Thus, our results demonstrate that the C-480T polymorphism in the HL promoter is associated with alterations in plasma lipids and lipoproteins and the accumulation of atherogenic LpC-III:B particles. (*Arterioscler Thromb Vasc Biol.* 1999;19:303-308.)

Key Words: hepatic lipase ■ gene promoter ■ polymorphisms ■ postprandial ■ lipoproteins

Hepatic lipase (HL) plays a role in the metabolism of several lipoproteins, thereby affecting plasma lipid levels.^{1,2} The plasma HL activity measured after heparin injection is inversely correlated with HDL cholesterol levels³ and postprandial lipoproteins.⁴ High HL activity is also associated with the occurrence of small, dense LDL particles.^{5,6} Several mechanisms may contribute to these associations. HL hydrolyzes phospholipids and triglycerides in LDL directly or after transfer to HDL, thus lowering LDL size. HDL phospholipid and triglyceride are preferred substrates for HL,⁷ and hydrolysis of phospholipid and triglyceride in HDL may induce cholesterol (ester) efflux to the lipase-containing tissues.⁸⁻¹¹ During this process HDL is converted to smaller particles, which can again take up cholesterol from peripheral tissues.^{8,12} In this way, HL is not only a determinant of HDL cholesterol levels but also may be an important element in reverse cholesterol transport. In humans, HL activity is also

correlated with postprandial lipid levels and may affect chylomicron remnant clearance.^{4,13} Inhibition of HL activity in rats by specific antibodies leads to impairment of chylomicron (remnant) clearance.¹⁴ The influence of HL on postprandial lipids may be direct or indirect. Hydrolysis of chylomicron remnant phospholipids by HL leads to the “unmasking” of apoE, thereby enhancing the binding of these particles to apoE-binding receptors.¹⁵ In addition, HL may act as a ligand for chylomicron remnants by binding the lipoproteins to receptors on the liver.¹⁶⁻¹⁸

Altogether, HL is an important enzyme with multiple functions affecting the metabolism, composition, and concentration of several lipoproteins. A deficiency of HL often leads to a mild phenotype with elevation of HDL and/or an increase in LDL concentration and buoyancy.^{13,19,20} In conjunction with other hyperlipidemia-causing genes, HL deficiency influences plasma lipoprotein levels more strongly, with tri-

Received January 28, 1998; revision accepted June 29, 1998.

From the Department of Internal Medicine III and Biochemistry, Erasmus University, Rotterdam, The Netherlands (H.J.); the Department of Medicine, Rayne Institute, University College Medical School, London, UK (G.C., P.J.T.); the Department of Biochemistry, National Public Health Institute, Helsinki, Finland (C.E.); Unité INSERM-325, Pasteur Institute, Lille, France (J.D.); and INSERM U258, Hôpital Broussais, Paris, France (V.N.).

Correspondence to: Hans Jansen, PhD, Department of Biochemistry, Ee 671, Erasmus University Rotterdam, POB 1738, 3000 DR Rotterdam, Netherlands. E-mail jansen@bc1.fgg.eur.nl

© 1999 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at <http://www.atvbaha.org>

glyceride enrichment of lipoprotein fractions with a $d > 1.006$ g/mL, the presence of β -VLDL, and impaired metabolism of postprandial triglyceride-rich lipoproteins.¹⁹ Thus, because of the multiple effects of HL on lipoprotein metabolism, genetic variation in HL expression may influence atherosclerotic risk.

Several variant sites have been identified in the HL gene, but only a few lead to decreased postheparin plasma HL activity.^{21–23} Recently, low HL activity was found to be associated with a common C-to-T base substitution in the HL promoter.^{24–27} This transition is at position -480 based on the sequence published by Cai et al²⁸ and at position -514 based on the sequence of Ameis and coworkers.²⁹ The -514 C-to-T polymorphism was recently shown to be in complete linkage disequilibrium with 3 other polymorphisms in the HL promoter (-250 G to A, -710 T to C, and -763 A to G).³⁰ To date no effects of any of these substitutions on HL promoter activity in vitro have been reported. In addition to low HL activity, the *T* allele was found to be associated with an increased HDL cholesterol content in males in the Netherlands, Finland, and the United States.^{25,31,30} In a Dutch sample of normolipidemic men with coronary artery disease (CAD), the frequency of the *T* allele was higher than in nonsymptomatic controls.²⁵ In contrast, in a Finnish study of subjects with a family history of myocardial infarction, the occurrence of the *T* allele was the same as in healthy subjects.³¹ We carried out our investigation on subjects taking part in the European Atherosclerosis Research Study (EARS) II, a postprandial trial of young, male, university students from 11 European countries (L. Tiret et al, unpublished observations, 1998) whose fathers had documented CAD (designated as cases) and age-matched controls, thus enabling us to examine populations of different European regions. Moreover, because HL deficiency may result in impaired postprandial lipoprotein metabolism,¹⁹ we studied whether the HL polymorphism influenced the lipid response to an oral fat load.

Methods

Subjects

Subjects were participants of EARS II, which was performed in 1993. Four hundred seven male students between the ages of 18 and 28 years whose fathers had proven myocardial infarction before the age of 55 (cases) were recruited from 14 university student populations from 11 European countries. One age-matched control was recruited by random selection from the same university populations ($n=415$). Tallinn, Estonia (cases/controls, 32/36) and Helsinki (32/33) and Oulu (23/23), Finland, were designated Baltic; Glasgow (31/31), Belfast (33/33), and Bristol (22/23) were designated United Kingdom; Aarhus, Denmark (30/30), Hamburg, Germany (32/32), Ghent, Belgium (32/32), and Zurich, Switzerland (36/36), were designated central Europe; and Lisbon, Portugal (18/18), Reus, Spain (30/33), Naples, Italy (30/30), and Athens, Greece (26/25), were designated southern Europe. The subjects were presumed to have been born in the country where they were studying. Details of lifestyle, smoking habits, alcohol consumption, medication, physical exercise, a personal and family history, and physiological measurements, were established using standardized questionnaires and protocols (Table 1).

Postprandial Tests

At the first visit each participant underwent a standard 75-g oral glucose tolerance test (OGTT) after a 12-hour overnight fast. Venous blood was withdrawn at 0, 30, 60, 90, and 120 minutes for the determination of glucose and insulin concentrations.

TABLE 1. Anthropometric and Lifestyle Characteristics in Cases and Controls

	Cases (n=385)	Controls (n=405)	P
Age, y	22.8 (0.1)	22.8 (0.1)	NS
Alcohol consumption, mL/d	20.1 (1.1)	18.0 (1.0)	NS
Smokers, %	26.6 (2.3)	25.0 (2.2)	NS
Physical activity, %			
Minimal	10.2 (1.6)	8.2 (1.4)	NS
Moderate	75.3 (2.2)	76.4 (2.1)	NS
Heavy	14.5 (1.8)	15.4 (1.8)	NS
Body mass index, kg/m ²	23.4 (0.1)	23.3 (0.1)	NS
Waist-hip ratio	0.849 (0.002)	0.851 (0.002)	NS
Triglycerides, mmol/L	1.00 (0.02)	0.96 (0.02)	NS
Total cholesterol, mmol/L	4.54 (0.04)	4.30 (0.04)	<0.001
HDL cholesterol, mmol/L	1.19 (0.01)	1.19 (0.01)	NS
LDL cholesterol, mmol/L	2.90 (0.04)	2.69 (0.04)	<0.001
ApoA-I, mg/dL	99.9 (0.9)	100.3 (0.8)	NS
ApoB, mg/dL	73.6 (0.9)	69.1 (0.8)	<0.001
ApoE, mg/dL	2.90 (0.04)	2.78 (0.04)	NS

Values are mean and (SD).

One week later the oral fat tolerance test was performed. The standard meal had an energy content of 1493 kcal (6186 kJ) consisting of 21.6 g protein, 65.5 g fat (of which 41.64 g was saturated), 56.2 g carbohydrate, and 416.6 mg cholesterol. The drink was made from 1 can of cream (Nestlé double cream), sugar (sucrose), and milk protein (Marvel). Blood samples were withdrawn at 0, 2, 3, 4, and 6 hours for the determination of triglyceride concentration. Levels of particles containing apoC-III and apoB (LpC-III:B) were measured at 0 and 4 hours.

Lipid and Lipoprotein Particle Measures

All fasting lipids and apolipoproteins were measured at baseline of the OGTT, and fasting triglycerides, insulin, and glucose were remeasured at baseline of the OGTT. LpC-III:B's were measured by a noncompetitive (sandwich) ELISA as described previously.³² In brief, polystyrene microtiter plates were coated with affinity-purified polyclonal antibodies to human apoC-III (1 mg/mL). Duplicate plasma samples were diluted 1:1000, 1:2000, and 1:5000 with 100 mmol/L PBS containing 1% albumin. The samples were added to the wells along with the standards and controls and incubated for 2 hours at 37°C. After incubation, the plates were washed 4 times with PBS, and a rabbit polyclonal apoB antibody conjugated to peroxidase was added. The plates were incubated for 2 hours at 37°C and then washed. Color development was performed for 30 minutes by addition of a peroxidase substrate (*o*-phenylenediamine dichloride, Sigma Chemical Co). The plates were read at 492 nm on an automated microplate reader model EL340 (Bio-Tek Instruments). A pool of 400 different plasma samples was used as a secondary standard. This pool was calibrated using immunopurified LpC-III:B. The apoB concentration of the particles was determined by nephelometry using the BNA system and apoB standards (Behringwerke). The values are expressed as the amount of apoB associated with apoC-III.

DNA Extraction and Genotyping

DNA was extracted by the salting-out method.³³ The DNA was stored diluted in 96-well Beckman plates,³⁴ divided into aliquots into 96-well Omnigene plates, and dried. The polymerase chain reaction (PCR) mixture consisted of 50 mmol/L KCl, 10 mmol/L Tris (pH 8.3), 0.1 g/L gelatin (Sigma), 0.2 mmol/L of each dNTP, 1.5 mmol/L MgCl₂, 0.02% W-1 (GIBCO), 0.1 U of *Taq* polymerase (Gibco BRL)/10 mL PCR, and 0.2 nmol/L of each of the PCR primers described by Jansen et al²⁵ for the C-480T variant.

TABLE 2. Genotype and Allele Frequencies of the C-480T Polymorphism of the HL Gene

	No.	T Allele Carriers, %	T Allele Frequency
All			
Cases	385	43.6	0.244
Controls	405	38.0	0.207
Baltic states			
Cases	85	45.9	0.241
Controls	90	47.8	0.261
UK			
Cases	76	40.8	0.237
Controls	85	35.3	0.200
Central Europe			
Cases	122	46.7	0.266
Controls	125	40.0	0.212
Southern Europe			
Cases	102	40.2	0.226
Controls	105	29.5	0.162

The PCR products were subjected to restriction enzyme analysis by digestion with 2 U of the restriction endonuclease *Nla*III (NEB) per 7 mL of the PCR sample at 37°C for 4 hours and in the buffer recommended by the manufacturer. The samples were then loaded on to an ethidium bromide–prestained microplate array diagonal gel electrophoresis gel as described,³⁴ electrophoresed for 1 hour and 20 minutes, and marked for the genotypes.

Statistical Analysis

The data were analyzed using the SAS statistical software package (SAS Institute Inc). Observed numbers of each genotype were compared with those expected for Hardy-Weinberg equilibrium in cases and controls from the 4 regions by using the χ^2 test. Allele frequencies were compared between cases and controls by the Mantel-Haenszel test after adjustment for region. Statistical significance for this and all other tests was taken to be $P < 0.05$. Triglycerides and LpC-III:B were logarithmically transformed before the tests to remove positive skewness. For postprandial analyses of triglycerides, 2 parameters were calculated: the area under the curve (AUC, in mmol-h/L) above the fasting concentration, calculated by the trapezoidal rule, and the peak (mmol/L), calculated as the highest value minus the fasting value. The C-480T genotype was tested after assuming additive effects of alleles in a codominant model. For lipid analysis, data for cases and controls were pooled because no significant interaction between case/control status and the HL -480 variant could be detected. The means were adjusted for status, age, and center.

Results

Study Population

The study population consisted of 790 subjects, of whom 385 were cases and 405 were controls. The cases and controls did not significantly differ in anthropometric or lifestyle characteristics. Total cholesterol, LDL cholesterol, and apoB were higher in cases than in controls (Table 1). The presence of the C-480T polymorphism was determined in all subjects. Overall, the frequency of the -480T allele was 0.207 in controls and 0.244 in cases ($P = 0.08$). The allele frequencies were in Hardy-Weinberg equilibrium in all 4 regions and are presented in Table 2.

TABLE 3. Plasma Lipids and Apolipoproteins According to the C-480T HL Promoter Polymorphism

	Genotype			Test*
	CC	CT	TT	
Cases:controls	217:251	148:140	20:14	
Triglyceride, mmol/L				
Cases	0.99 (0.03)	1.02 (0.04)	1.02 (0.10)	<0.05
Controls	0.93 (0.03)	0.99 (0.04)	1.13 (0.11)	
Cholesterol, mmol/L				
Cases	4.47 (0.06)	4.62 (0.07)	4.56 (0.18)	<0.01
Controls	4.23 (0.05)	4.40 (0.07)	4.88 (0.22)	
LDL cholesterol, mmol/L				
Cases	2.88 (0.05)	2.96 (0.06)	2.84 (0.16)	NS
Controls	2.65 (0.05)	2.73 (0.06)	2.89 (0.20)	
HDL cholesterol, mmol/L				
Cases	1.16 (0.02)	1.21 (0.02)	1.27 (0.05)	0.01
Controls	1.16 (0.02)	1.22 (0.02)	1.25 (0.08)	
ApoA-I, mg/dL				
Cases	97.9 (1.1)	101.8 (1.4)	108.1 (3.8)	<0.001
Controls	98.0 (1.1)	103.6 (1.4)	108.5 (4.5)	
ApoB, mg/dL				
Cases	72.5 (1.1)	75.5 (1.4)	71.8 (3.7)	<0.05
Controls	67.8 (1.1)	70.8 (1.4)	74.8 (4.4)	
ApoE, mg/dL				
Cases	2.87 (0.06)	2.92 (0.07)	3.04 (0.19)	NS
Controls	2.74 (0.05)	2.82 (0.07)	3.11 (0.22)	

*The P values refer to the levels of significance in the different variables after pooling the data for cases and controls.

Relation to Plasma Lipids, Lipoproteins, and Apolipoproteins

The association of the rare -480T allele with plasma lipid and lipoprotein levels in cases and controls is shown in Table 3. There was no significant interaction between case/control status and genotype, so cases and controls were pooled to acquire sufficient numbers for T/T carriers. In the pooled data, the -480T allele was associated with higher fasting plasma total cholesterol, triglyceride, and HDL cholesterol. These results conform to a codominant model, because the homozygous T/T carriers had the highest values followed by the C/T carriers, and the lowest values were found in the C/C homozygotes. LDL cholesterol did not differ among the different genotypes. Although in carriers of the T allele both plasma triglyceride and HDL were increased, the inverse correlation between fasting triglyceride level and HDL cholesterol concentration was significantly stronger in the -480T homozygotes than in heterozygotes or -480C homozygotes (correlation coefficients of -0.65, -0.26, and -0.32, respectively; $P < 0.05$). The strongest association of the C-480T polymorphism was with apoA-I. In T/T carriers the apoA-I concentration was 10% higher than in -480C/ C homozygotes ($P < 0.001$). ApoB was slightly higher in T allele carriers than in C/C homozygotes, but there was no

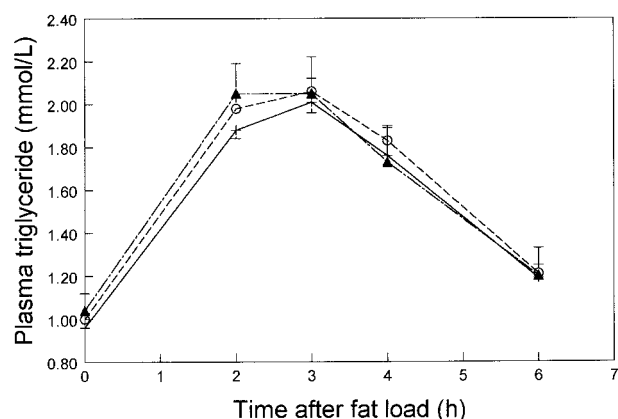


Figure 1. Postprandial changes in plasma triglyceride concentration after the fat tolerance test according to C-480T polymorphism. Mean plasma triglyceride concentrations \pm SD are given. --- indicates CC genotype; -○-, CT genotype; and -▲-, TT genotype. Values are adjusted for age, center, and case/control status. All associations were not significant, whether tested as 3 codominant classes or *T*+ versus CC. There were no significant interactions with status, body mass index, waist-hip ratio, or fasting triglyceride level.

difference between the *C/T* and *T/T* genotypes. There was no genotype effect on body mass index, waist-hip ratio, or plasma glucose or insulin concentrations.

Postprandial Triglyceride and LpC-III:B Response

Because HL is involved in postprandial lipid clearance, we studied the effects of the C-480T polymorphism on postprandial triglyceride concentrations after a fat tolerance test (Figure 1). There were no significant differences in the mean triglyceride concentrations between the different genotypes when tested either directly or after adjustment for fasting triglyceride. Differences in the AUC, peak height minus fasting concentration, or the time at which the triglyceride value reached its peak did not reach statistical significance among genotypes (Table 4). The association of the polymorphism with the concentration of LpC-III:B was also studied before and after the fat load. Before the fat load, the concentration of LpC-III:B was dose-dependently higher in

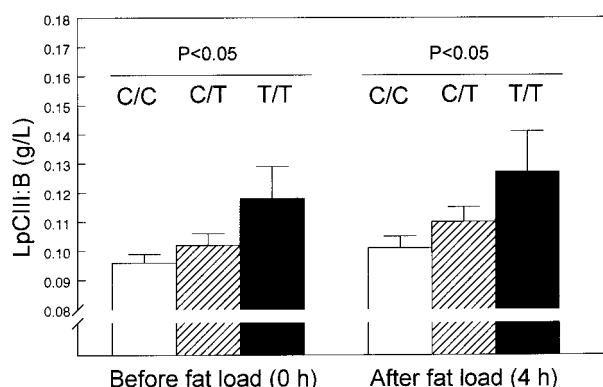


Figure 2. LpC-III:B concentrations according to C-480T polymorphism. LpC-III:B concentrations in different genotypes were measured before and 4 hours after an oral fat load. Open bars indicate *C/C* genotype (*n*=395); hatched bars, *C/T* genotype (*n*=244); and solid bars, *T/T* genotype (*n*=32). Values are means adjusted for age, center, and case/control status. C-480T was tested after assuming codominance. The test was performed on logarithmically transformed values. There were no significant interactions with status, body mass index, or waist-hip ratio.

the -480T allele carriers than in noncarriers. Homozygous *T* allele carriers had a 23% higher value than did individuals homozygous for the -480C allele ($P<0.05$; Figure 2). After the fat load this difference in LpC-III:B concentration remained, with -480T homozygotes having the highest levels of LpC-III:B ($P<0.05$).

Discussion

We determined the frequency of the *T* allele of the HL gene promoter polymorphism, referred to as C-480T²⁴ or -514C/T,³⁰ and lipid associations in young, healthy men whose fathers had had a premature myocardial infarction (cases) and in age-matched controls (EARS II; L. Tiret et al, unpublished observations, 1998). Overall, the allele frequencies found were in the same range (≈ 0.2) as those previously reported in the Netherlands, Finland, and the United States.^{25,30,31} The study population consisted of university students recruited in northern (the Baltic), central, and southern Europe and the United Kingdom. When all 4 regions were pooled, no significant difference in the frequency of the *T* allele between cases and controls was found ($P=0.08$). The *T* allele was associated with an increased concentration in plasma triglyceride, total cholesterol, HDL cholesterol, apoA-I, and apoB. Whether or to what extent the changes in plasma lipids are related to atherosclerosis is not clear. On the one hand, HDL cholesterol and apoA-I were increased in *T* allele carriers, suggesting that the *T* allele may be associated with a reduced atherogenic risk. On the other hand, the *T* allele was also associated with higher plasma triglyceride and cholesterol. Moreover, in carriers of the *T* allele, the concentration of LpC-III:B was significantly increased both preprandially and postprandially. ApoC-III is an important determinant of triglyceride-rich lipoprotein clearance. In vitro studies have shown that apoC-III inhibits lipoprotein lipase³⁵ and HL activities³⁶ and displaces apoE from the surface of triglyceride-rich lipoproteins. Therefore, in metabolic conditions in which lipolytic activity is decreased, lipoprotein particles that are enriched in apoC-III tend to accumulate. LpC-III:B is associated with CAD.³⁷⁻⁴⁰ Thus overall, the *T* allele is

TABLE 4. Plasma Triglyceride Concentration and LpC-III:B After the Fat Tolerance Test According to C-480T Genotype

	Genotype			<i>P</i> *
	<i>CC</i>	<i>CT</i>	<i>TT</i>	
Cases:controls	207:237	137:129	17:14	
AUC†	3.82 (0.11)	3.99 (0.14)	3.71 (0.41)	NS
Peak height minus fasting value†	1.20 (0.03)	1.25 (0.04)	1.20 (0.12)	NS
Time to peak‡	2.91 (0.04)	2.92 (0.05)	2.52 (0.16)	NS
Cases:controls	184:211	129:115	18:14	
LpC-III:B, g/L				
0 Hour	0.096 (0.003)	0.102 (0.004)	0.118 (0.011)	<0.05
4 Hours	0.101 (0.004)	0.110 (0.005)	0.127 (0.014)	<0.05

*The *P* values refer to the levels of significance in the different variables after pooling the data for cases and controls.

†Test performed on square-root values.

‡Wilcoxon rank-sum test.

associated with a number of potentially antiatherogenic and proatherogenic lipoprotein changes in our population. In a population of normolipidemic men with CAD²⁵ and in white Americans,³⁰ the *T* allele was also associated with enhanced HDL, but not with total cholesterol and triglyceride. In our population, why effects of the *T* allele on total cholesterol and triglyceride were also found is not clear. Possibly, differences in selection criteria (age, normolipidemia) between the different studies may play a role. Although we did not determine HL activity in our population, the changes in plasma lipids are presumably related to a lower HL activity in the *T* allele carriers, as has been shown to exist in a number of other populations.^{25–27} In which way the C–480T substitution or other polymorphisms lead to lowered HL expression is not known. To date, no effects of any of these substitutions on HL promoter activity in vitro have been reported. It is interesting to note that the C-to-T substitution at –480 disrupts a potential upstream stimulatory factor binding site. Upstream stimulatory factor is an insulin-inducible transcription factor involved in the regulation of hepatic lipid and carbohydrate metabolism.^{41–43} However, whether this is related to or causes the lowered HL activity in the *T* allele carriers is not clear. Studies on this subject are in progress.

Appendix

EARS II Project Leader

D. St. J. O'Reilly, UK

EARS II Project Management Group

F. Cambien, France; G. De Backer, Belgium; D. St. J. O'Reilly, UK; M. Rosseneu, Belgium; J. Shepherd, UK; L. Tiret, France.

The EARS II Group Collaborating Centers and Their Associated Investigators

Austria: H.J. Menzel, Institute for Medical Biology and Genetics, University of Innsbruck, laboratory.

Belgium: G. De Backer, S. De Henauw, Department of Public Health, University of Ghent, recruitment center; M. Rosseneu, Laboratorium voor Lipoproteïne Chemie/Vakgroep Biochemie, University of Ghent, laboratory.

Denmark: O. Faergeman, C. Gerdes, Medical Department I, Aarhus Amtssygehus, Aarhus, recruitment center.

Estonia: M. Saava, Department of Nutrition and Metabolism, Institute of Cardiology, Tallinn, recruitment center.

Finland: C. Ehnholm, National Public Health Institute, and R. Elovainio, J. Peräsalu, Finnish Student Health Service, Helsinki, recruitment center; Y.A. Kesäniemi, M.J. Savolainen, Department of Internal Medicine and Biocenter Oulu, and P. Palomaa, Finnish Student Health Service, University of Oulu, Oulu, recruitment center and laboratory.

France: L. Tiret, V. Nicaud, J. Boer, R. Rakotovaio, INSERM U258, Hôpital Broussais, Paris, EARS data center; S. Visvikis, Center de Médecine Préventive, Nancy, laboratory; J.C. Fruchart, J. Dallongeville, Service de Recherche sur les Lipoprotéines et l'Athérosclérose (SERLIA), INSERM U325, Institut Pasteur, Lille, laboratory.

Germany: U. Beisiegel, C. Dingler, Medizinische Klinik Universitäts-Krankenhaus Eppendorf, Hamburg, recruitment center and laboratory.

Greece: G. Tsitouris, N. Papageorgakis, Department of Medicine and Cardiology, Evangelismos Hospital, Athens, recruitment center.

Italy: E. Farinero, Institute of Internal Medicine and Metabolic Disease, University of Naples, Naples, recruitment center.

The Netherlands: L.M. Havekes, IVVO-TNO Health Research, Gaubius Institute, Leiden, laboratory.

Portugal: M.J. Halpern, J. Canena, Instituto Superior de Ciencias da Saude, Lisbon, recruitment center.

Spain: L. Masana, J. Ribalta, Unitat Recerca Lipids, University Rovira i Virgili, Reus, recruitment center and laboratory.

Switzerland: F. Gutzwiller, B. Martin, Institute of Social and Preventive Medicine, University of Zurich, Zurich, recruitment center and laboratory.

United Kingdom: D. St. J. O'Reilly, M. Murphy, Institute of Biochemistry, Royal Infirmary, Glasgow, recruitment center and laboratory; S. Humphries, P. Talmud, V. Gudnason, R. Fisher, University College London School of Medicine, London, laboratory; D. Stansbie, A.P. Day, M. Edgar, Department of Chemical Pathology, Royal Infirmary, Bristol, recruitment center and laboratory; F. Kee, Northern Health and Social Services Board, and A. Evans, Department of Epidemiology and Public Health, Queen's University of Belfast, Belfast, recruitment center.

Acknowledgments

This work was supported by the European Community (EU-BIOMED 2, BMG4-98-3324). P.J.T. is supported by the British Heart Foundation.

References

- Jansen H, Hülsmann WC. Enzymology and physiological role of hepatic lipase. *Biochem Soc Trans.* 1985;13:24–26.
- Applebaum-Bowden D. Lipases and lecithin:cholesterol acyltransferase in the control of lipoprotein metabolism. *Curr Opin Lipidol.* 1995;6:130–135. Review.
- Kuusi T, Saarinen P, Nikkila EA. Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoprotein, in man. *Atherosclerosis.* 1980;36:589–593.
- De Bruin TWA, Brouwer CB, Gimpel JA, Erkelens DW. Postprandial decrease in HDL cholesterol and HDL apo A-I in normal subjects in relation to triglyceride metabolism. *Am J Physiol.* 1991;260:E492–E498.
- Jansen H, Hop W, van Tol A, Bruschke AVG, Birkenhäger JC. Hepatic lipase and lipoprotein lipase are not major determinants of the low density lipoprotein subclass pattern in human subjects with coronary heart disease. *Atherosclerosis.* 1994;107:45–54.
- Tan KCB, Cooper MB, Ling KLE, Griffin BA, Freeman DJ, Packard CJ, Shepherd J, Hales CN, Betteridge DJ. Fasting and postprandial determinants for the occurrence of small dense LDL species in non-insulin-dependent diabetic patients with and without hypertriglyceridaemia: the involvement of insulin, insulin-precursor species and insulin resistance. *Atherosclerosis.* 1995;113:273–287.
- Groot PHE, Jansen H, van Tol A. Selective degradation of the high density lipoprotein-2 subfraction by heparin-releasable liver lipase. *FEBS Lett.* 1981;129:269–272.
- Jansen H, Hülsmann WC. Heparin-releasable (liver) lipase(s) may play a role in the uptake of cholesterol by steroid-secreting tissues. *Trends Biochem Sci.* 1980;5:265–268.
- Johnson WJ, Bamberger MJ, Latta RA, Rap PE, Phillips MC, Rothblat GH. The bidirectional flux of cholesterol between cells and lipoproteins: effects of phospholipid depletion of high density lipoprotein. *J Biol Chem.* 1986;261:5766–5776.
- Kadowaki H, Patton GM, Robins SJ. Metabolism of high density lipoprotein lipids by the rat liver: evidence for participation of hepatic lipase in the uptake of cholesteryl ester. *J Lipid Res.* 1992;33:1689–1698.
- Marques-Vidal P, Azéma C, Collet X, Vieu C, Chap H, Perret B. Hepatic lipase promotes the uptake of HDL esterified cholesterol by the perfused rat liver: a study using reconstituted HDL particles of defined phospholipid composition. *J Lipid Res.* 1994;35:373–384.
- Clay MA, Newnham HH, Forte TM, Barter PJ. Cholesteryl ester transfer protein and hepatic lipase activity promote shedding of apo A-I from HDL and subsequent formation of discoidal HDL. *Biochim Biophys Acta.* 1992;1124:52–58.
- Hegele RA, Little JA, Vezina C, Maguire GF, Tu L, Wolever TS, Jenkins DJA, Connelly PW. Hepatic lipase deficiency: clinical, biochemical, and molecular genetic characteristics. *Arterioscler Thromb.* 1993;13:720–728.
- Sultan F, Lagrange D, Jansen H, Griglio S. Inhibition of hepatic lipase activity impairs chylomicron remnant-removal in rats. *Biochim Biophys Acta.* 1990;1042:150–152.
- Borenstajn J, Getz GS, Kotlar TJ. Uptake of chylomicron remnants by the liver: further evidence for the modulating role of phospholipids. *J Lipid Res.* 1988;29:1087–1096.
- Krapp A, Ahle S, Kersting S, Hua Y, Kneser K, Nielsen M, Gliemann J, Beisiegel U. Hepatic lipase mediates the uptake of chylomicrons and

- β -VLDL into cells via the LDL receptor-related protein (LRP). *J Lipid Res.* 1996;37:926-936.
17. Ji Z-S, Lauer SJ, Fazio S, Bensadoun A, Taylor JM, Mahley RW. Enhanced binding and uptake of remnant lipoproteins by hepatic lipase-secreting hepatoma cells in culture. *J Biol Chem.* 1994;269:13429-13436.
 18. Shafi S, Brady SE, Bensadoun A, Havel RJ. Role of hepatic lipase in the uptake and processing of chylomicron remnants in rat liver. *J Lipid Res.* 1994;35:709-720.
 19. Connelly PW, Maguire GF, Lee M, Little JA. Plasma lipoproteins in familial hepatic lipase deficiency. *Arteriosclerosis.* 1990;10:40-48.
 20. Breckenridge WC, Little JA, Alaupovic P, Wang CS, Kuksis A, Kakis G, Lindgren F, Gardiner G. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis.* 1982;45:161-179.
 21. Brand K, Dugi KA, Brunzell JD, Nevin DN, Santamarina-Fojo S. A novel A→G mutation in intron I of the hepatic lipase gene leads to alternative splicing resulting in enzyme deficiency. *J Lipid Res.* 1996;37:1213-1223.
 22. Knudsen P, Antikainen M, Ehnholm S, Uusi-Oukari M, Tenkanen H, Lahdenperä S, Kahri J, Tilly-Kiesi M, Bensadoun A, Taskinen M-R, Ehnholm C. A compound heterozygote for hepatic lipase gene mutations Leu334→Phe and Thr383→Met: correlation between hepatic lipase activity and phenotypic expression. *J Lipid Res.* 1996;37:825-834.
 23. Hegele RA. The molecular basis of hepatic lipase deficiency. *Can Med Assoc J.* 1991;145:1277.
 24. Jansen H, Verhoeven AJM, Halley DJJ, Kastelein JJP. C-T substitution at -480 of the hepatic lipase (HL) promoter associated with a lowered HL activity. Florence, Italy: 66th Congress of the European Atherosclerosis Society. 1996:20. Abstract.
 25. Jansen H, Verhoeven AJM, Weeks L, Kastelein JJP, Halley DJJ, van den Ouweland A, Jukema JW, Seidell JC, Birkenhäger JC. A common C to T substitution at position -480 of the hepatic lipase promoter associated with a lowered lipase activity in coronary artery disease patients. *Arterioscler Thromb Vasc Biol.* 1997;17:2837-2842.
 26. Tahvanainen E, Syväne M, Frick MH, Murtomäki-Repo S, Antikainen M, Kesäniemi YA, Kauma H, Pasternak A, Taskinen M-R, Ehnholm C, for the LOCAT Study Investigators. Association of variation in hepatic lipase activity with promoter variation in the hepatic lipase gene. *J Clin Invest.* 1998;101:956-960.
 27. Vega GL, Clark LT, Tang A, Marcovina S, Grundy SM, Cohen JC. Hepatic lipase activity is lower in African American men than in white American men: effects of 5' flanking polymorphism in the hepatic lipase gene (LIPC). *J Lipid Res.* 1998;39:228-232.
 28. Cai S-J, Wong DM, Chen S-H, Chan L. Structure of the human hepatic triglyceride lipase gene. *Biochemistry.* 1989;28:8966-8971.
 29. Ameis D, Stahnke G, Kobayashi J, McLean J, Lee G, Büscher M, Schotz MC, Will H. Isolation and characterization of the human hepatic lipase gene. *J Biol Chem.* 1990;265:6552-6555.
 30. Guerra R, Wang J, Grundy SM, Cohen JC. A hepatic lipase (LIPC) allele associated with high plasma concentrations of high density lipoprotein cholesterol. *Proc Natl Acad Sci U S A.* 1997;94:4532-4537.
 31. Murtomäki S, Tahvanainen E, Antikainen M, Tiet L, Nicaud V, Jansen H, Ehnholm C. Hepatic lipase gene polymorphisms influence plasma HDL levels: results from Finish EARS participants. *Arterioscler Thromb Vasc Biol.* 1997;17:1879-1884.
 32. Kandoussi A, Cachera C, Parsy D, Bard JM, Fruchart J-C. Quantitative determination of different apolipoprotein B containing lipoproteins by an enzyme linked immunosorbent assay. *J Immunoassay.* 1991;12:305-323.
 33. Bolla MK, Haddad L, Humphries SE, Winder AF, Day INM. High-throughput method for determination of apoE genotypes with use of restriction digestion analysis by microplate array diagonal gel electrophoresis. *Clin Chem.* 1995;41:1599-1604.
 34. Day INM, Humphries SE. Electrophoresis for genotyping: microtitre array diagonal gel electrophoresis (MADGE) on horizontal polyacrylamide (H-PAGE) gels, Hydrolink or agarose. *Anal Biochem.* 1994;222:389-395.
 35. McConathy WJ, Gesquiere JC, Bass H, Tartar A, Fruchart J-C, Wang C-S. Inhibition of lipoprotein lipase activity by synthetic peptides of apolipoprotein C-III. *J Lipid Res.* 1992;33:995-1003.
 36. Kinnunen PKJ, Ehnholm C. Effect of serum and apoC-apoproteins from very low density lipoproteins on human postheparin plasma hepatic lipase. *FEBS Lett.* 1976;65:354-357.
 37. Luc G, Fievet C, Arveiler D, Evans AE, Bard J-M, Cambien F, Fruchart J-C, Ducimetiere P. Apolipoproteins C-III and E in apoB- and non-apoB-containing lipoproteins in two populations at contrasting risk for myocardial infarction: the ECTIM study: Etude Cas Témoin sur Infarctus du Myocarde. *J Lipid Res.* 1996;37:508-517.
 38. Björkegren J, Hamsten A, Milne RW. Alterations of VLDL composition during alimentary lipemia. *J Lipid Res.* 1997;38:301-314.
 39. Blankenhorn DH, Alaupovic P, Wickham E, Chin HP, Azen SP. Prediction of angiographic change in native human coronary arteries and aortocoronary bypass grafts: lipid and nonlipid factors. *Circulation.* 1990;81:470-476.
 40. Hodis HN, Mack WJ, Azen SP, Alaupovic P, Pogoda JM, LaBree L, Hemphill LC, Kramsch DM, Blankenhorn DH. Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastatin. *Circulation.* 1994;90:42-49.
 41. Wang D, Sul HS. Upstream stimulatory factor binding to the E-box at -65 is required for insulin regulation of the fatty acid synthase promoter. *J Biol Chem.* 1997;272:26367-26374.
 42. Girard J, Ferre P, Foufelle F. Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. *Annu Rev Nutr.* 1997;17:325-352.
 43. Kahn A. Transcriptional regulation by glucose in the liver. *Biochimie.* 1997;79:113-118.